ISSN 0704-3716

Canadian Translation of Fisheries and Aquatic Sciences

No. 5423

The possibility of obtaining reproductive products and larvae of bivalve mollusks by hormonal stimulation within a specified period

S. M. Nikitina, N. P. Kudikina, N. V. Chibisova,
O. O. Golovina, and T. G. Lvova

Original title: K voprosu o vozmozhnosti polucheniya polovykh produktov i lichinok dvustvorchatykh mollyuskov v kontroliruyemyye sroki pod vozdeistviyem gormonal'nykh preparatov

In: Biology and cultivation of mollusks (Biologiya i kul'tivirovaniye mollyuskov). Moscow, Sb. Nauchn. Tr. VNIRO, 1987. p. 24-32

Original language: Russian

Available from:
Canada Institute for Scientific and Technical Information
National Research Council
Ottawa, Ontario, Canada K1A 0S2

1988

13 typescript pages

Secretary of State / Secrétariat d'État

90-01523/

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU BUREAU DES TRADUCTIONS

LIBRARY IDENTIFICATION – FICHE SIGNALÉTIQUE

Translated from - Traduction de	Into - En
Russian	English

Author - Auteur

S.M. Nikitina et al.

Title in English or French - Titre anglais ou français

The possibility of obtaining reproductive products and larvae of bivalve mollusks by hormonal stimulation within a specified period

Title in foreign language (Transliterate foreign characters)
Titre en langue étrangère (Transcrire en caractères romains)

K voprosu o vozmozhnosti polucheniya polovykh produktov i lichinok dvustvorchatykh mollyuskov v kontroliruyemyye sroki pod vozdeistviyem gormonal'nykh preparatov

Reference in foreign language (Name of book or publication) in full, transliterate foreign characters.
Référence en langue étrangère (Nom du livre ou publication), au complet, transcrire en caractères romains.

Biologiya i kul'tivirovaniye mollyuskov

Reference in English or French - Référence en anglais ou français

Biology and Cultivation of Mollusks

Publisher - Éditeur	DATE OF PUBLICATION / DATE DE PUBLICATION			Page Numbers in original / Numéros des pages dans l'original
not available	Year / Année	Volume	Issue No. / Numéro	24-32
Place of Publication / Lieu de publication				Number of typed pages / Nombre de pages dactylographiées
Moscow, USSR	1987	–	–	11

Requesting Department / Ministère-Client: D F O

Translation Bureau No. / Notre dossier nº: 3038751

Branch or Division / Direction ou Division: Scientific Publications

Translator (Initials) / Traducteur (Initiales): N. De.

Person requesting / Demandé par: Neil Bourne

Your Number / Votre dossier nº: ---

Date of Request / Date de la demande: August 23, 1988

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISÉE
Information seulement

OCT 21 1988

Canada

SEC 5-111 (84-10)

Secretary of State | Secrétariat d'État

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU | BUREAU DES TRADUCTIONS

Client's No.–N° du client	Department – Ministère	Division/Branch – Division/Direction	City – Ville
—	D F O	Scientific Publications	Ottawa
Bureau No.–N° du bureau	**Language – Langue**	**Translator (Initials) – Traducteur (Initiales)**	
3038751	Russian	N. De.	OCT 21 1988

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISÉE
Information seulement

Biologiya i kul'tivirovaniye mollyuskov (Biology and Cultivation of Mollusks), 1987, pp. 24-32. Moscow

(24)*

The possibility of obtaining reproductive products and larvae of bivalve mollusks by hormonal stimulation within a specified period

by S.M. Nikitina, N.P. Kudikina, N.V. Chibisova, O.O. Golovina and T.G. Lvova

(Kaliningrad State University)

Knowledge of the endocrine regulation of seasonal biological phenomena (reproduction, moulting, etc.) in invertebrates is highly important to the development of the biotechnology of breeding commercially promising species.

(25)

Some steroidal and peptide hormones affect the reproduction of vertebrates and in some cases invertebrates (Malitsa, 1979; Khotimchenko, 1977, 1978; Mori, 1979; Bomirski, Klek-Kawinska, 1976; Takahashi, 1982; Kulkarni et al., 1981). It is possible that this intensifies the metabolic processes in cultivated animals and their reproductive potentials are realized to a fuller extent as a result.

Our investigations during 1981-1983 have shown that the use of hormonal preparations can affect the reproduction of invertebrates (Endocrine regulation..., 1981; Problems of hormonal regulation..., 1982, 1983; Nikitina, 1982; Nikitina, Chibisova,

Canada

*The numbers in the right-hand margin are the pages of the Russian text - transl.

1982; Nikitina et al., 1984). However, not all of the hormones by far have been studied in this respect. This particular investigation was carried out in order to study the stimulating effect of various hormonal preparations on the spawning of the Black Sea mussel, Mytilus galloprovincialis Lam.

Material and Method

The subject of study consisted of Black Sea mussels of the species Mytilus galloprovincialis Lam., taken from piles 50 m from shore at a depth of 3-5 m. The experiments were carried out during June—October 1984 at the VNIRO* Experimental Station near the village of B. Utrish. During our study of the effectiveness of hormonal preparations and their combinations in stimulating spawning (see table), we performed three experiments on animals of the same size—weight group.

In the first experiment, we studied the one-time effect of the hormonal preparations and their combinations on 26 groups of animals taken from a natural habitat and found at different stages of the sexual cycle. The combined effect of the hormones was achieved by repeated injections at intervals of not more than 2 h. In the second and third experiments, mussels of the same group were injected with the hormonal preparations 2 and 3 times respectively at certain time intervals (from 2 to 6 weeks). The second experiment included five groups of animals, and the third experiment two groups. During the period between stimulations, the mussels were kept in cages fastened to the piles in the bay (depth 3-5 m). The control in all three experiments consisted of individuals taken from a natural habitat, which had been given a placebo injection.

*All-Union Scientific Research Institute of Sea Fisheries and Oceanography - transl.

During the experiments, the groups of control and test animals were kept in aquariums containing seawater throughout the period of spawning stimulation. The stimulating effect of this or that hormonal preparation was judged by the number of spawning individuals and the quantity of spawned reproductive products. The activity of spawning (A) was determined by the formula $A=n/n_o$, where n denotes the number of spawning individuals, and n_o the number of individuals in the experiment. (26)

Hormonal preparations used in the experiments

Preparation	Manufacturer	Dose per individual
Hydrocortisone	"Gedeon Richter" chemical plant (Budapest, Hungary)	2.5 μg
Prednisolone	Ditto	3.0 μg
Propionate testosterone	Rostov-on-Don Chemical Plant	5.0 μg
Progesterone	Ditto	2.5 μg
Folliculin (estrone)	Kaunas Plant for Endocine Preparations	0.05 μg
Prephyson* (standard extract of the prehypophysis)	PROMONTA (Hamburg)	2.5 ME
Chorionic gonadotropin	Moscow plant for Endocrine Preparations	0.5 ME
Pituitrin (posthypophyseal extract from cattle)	Kaunas Plant for Endocrine Preparations	0.5 ME
Mammophysin*	Ditto	0.5 ME

*conjectural spelling - transl.

The number of egg cells spawned by one female was counted by the standard method with the help of a Bogorov chamber. Observations of cleavage and the formation of the blastula, early trochophore and veliger larva were carried out every 10-15 minutes for the first two hours of spawning, every 30-60 min for the following

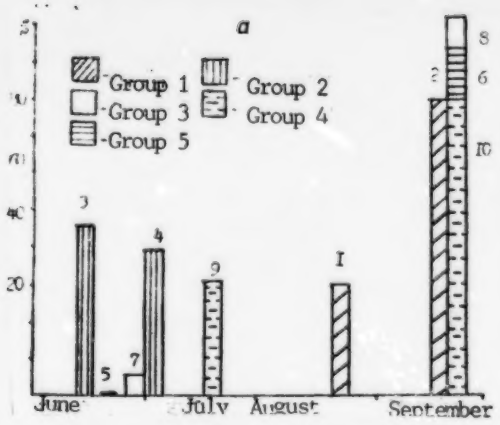
three hours, and once every 24 hours up to the end of the experiment.

Results

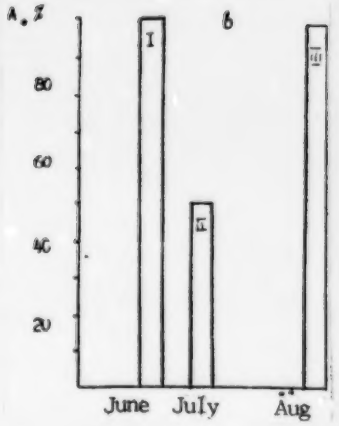
Our study of the one-time effect of certain hormonal preparations and their combinations on spawning (first experiment carried out during June—October 1984) has shown that the highest percentage of spawners in June-July is achieved with injections of prednisolone (80%) and a combination of folliculin and prephyson (75%). Only 25% of the animals spawned when prephyson was injected. Injections of folliculin and chorionic gonadotropin achieved practically the same percentage of spawning individuals (5-10%). (27)

The experiments in August showed that the combined use of folliculin and prephyson resulted in 30, 60 and 80% spawning in August, September and October respectively. The stimulation of mussel spawning during this period showed that practically all of the hormonal preparations and combinations used were effective stimulants of spawning, except for chorionic gonadotropin and the combination of folliculin, prephyson and pituitrin, which produced a mere 10% spawning effect.

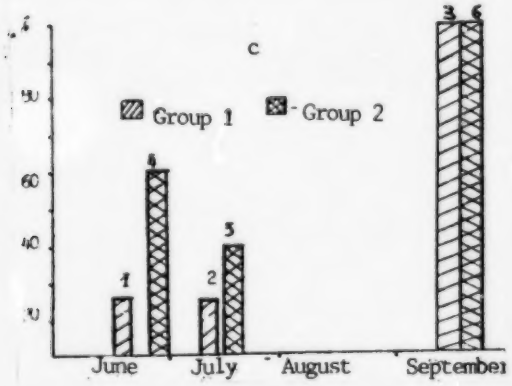
The injections of hormonal preparations stimulated spawning in more than 50% of the animals involved in the experiments. 100% spawning was observed in the experiment with one-time injection of prephyson, and 95% spawning was achieved with pituitrin and prednisolone. 65-75% spawning was noted under the effect of folliculin, 75-85% spawning with progesterone, 90% with mammophysin, and 50-75% spawning with testosterone.



1,3,4,6,8 - injections of folliculin and prephyson; 2,9,10 - injections of folliculin; 5 - addition of hydrocortisone to the water; 7 - injection of chorionic gonadotropin and addition of the same to the water



I - temperature stimulation, injection of prephyson and pituitrin; II, III - injection of folliculin and prephyson



1,2 - injection of hydrocortisone;
3,5,6 - injection of folliculin;
4 - injection of prephyson

Changes in the activity of spawning (%) under the effect of individual hormonal preparations and their combinations with two-time (a) and three-time (b) injection, as well as with three-time injection at a low water temperature (c)

Our study of the effect of hormonal preparations and their combinations in the second experiment points to the fact that the degree of reaction to the first and second effects is determined by the properties of the hormonal preparations and the duration of the experiment.

Folliculin combined with prephyson was injected in the 1st group of animals on 25 August 1984. The percentage of spawners was not very high, which was probably due to the fact that the reproductive products had been cast during natural spawning. The injection of folliculin on September 18 stimulated spawning in 80% of the animals (see diagram a).

The folliculin injected in the 4th group of animals on July 19 causedonly 30% of the mussels to spawn. A second injection of the same given during the same period as in the first group of animals (September 29) induced spawning in 80% of the mussels studied. A high percentage of spawning individuals was also noted when a second injection of folliculin combined with prephyson was given (5th and 3rd groups). However, the number of spawners depends on the properties of the hormonal preparation used during the first stimulation of spawning. 100% spawning was noted in the case where a previous injection of chorionic gonadotropin had been administered (3rd group). The effect of a second stimulation of spawning with folliculin combined with prephyson 18 days after the first stimulation (July 3rd, 2nd group) was lower (36% spawning, i.e. fewer than with one-time stimulation when 45% of the individuals spawned). (28)

With three-time injection of hormonal preparations and their combinations, the maximum number of spawning mussels was noted, (29)

as in the case of two-time injection, when the animals of both of the study groups were injected with folliculin (see diagram c). When hydrocortisone was injected once in the 1st group of mussels, the number of spawners was quite low, 15% of the total number of experimental animals. A second injection of hydrocortisone had a similar effect. Prephyson was injected as the first stimulant of spawning in the 2nd group of animals; unlike hydrocortisone, it had a more pronounced stimulating effect. The number of spawners constituted 50%. The folliculin injected 20 days later reduced the percentage of spawners somewhat.

Of special interest is the group of mussels which were kept at a low temperature from March 1984 up to their inclusion in the experiment, and did not spawn (see diagram b). A gradual increase in temperature caused 50% of these mussels to spawn. Subsequent injections of prephyson and pituitrin increased the number of spawning individuals to 100%. An injection of folliculin combined with prephyson 20 days after the first injection induced spawning in 50% of the mussels. The decrease in the number of spawning animals may be due to the fact that the gonads of the mussels had not yet undergone complete restoration after the first spawning. When folliculin and prephyson were injected one month after the second injection, we again noted a high percentage of spawners (97.8%), i.e. it took 50 days to achieve 100% in this group of mussels.

Another indication of the efficacy of individual hormonal preparations or their combinations is the number of reproductive products cast out during stimulated spawning, which, in turn, is

determined by the physiological condition of the spawners.

Six of the eight preparations used, namely testosterone, folliculin, pituitrin, prednisolone, mammophysin and prephyson, had approximately the same stimulating effect on the spawners of Mytilus galloprovincialis. The number of egg cells spawned by the females of this species after stimulation with pituitrin and prednisolone constituted 1,500,000. The stimulating effect of mammophysin, folliculin and testosterone was less significant (1,250,000 egg cells). The effect of the hormonal preparations diminished in the following order: prephyson (800,000 egg cells), progesterone (600,000 egg cells) and chorionic gonadotropin (300,000 egg cells). The control animals, which were given a placebo injection, did not spawn.

When folliculin was used in a combination with prephyson to stimulate spawning, fewer gametes were cast out than when folliculin was used on its own. On the other hand, when these hormones are used after injections of hydrocortisone and chorionic gonadotropin, the effect is more significant.

The maximum number of gametes (3,200,000) was observed when a combination of prephyson and pituitrin was used as the spawning stimulant. However, this should be regarded as a special case, since here we used adult fish that did not take part in spawning (non-spawning) and were, therefore, at another stage of gametogenesis. During the second hormonal stimulation of this group one month after the first one mentioned above, we noted a substantial decrease in the intensity of spawning compared with the first time, though it remained quite high (about 2,000,000 gametes per female).

The development of early larvae, achieved by artificial fertilization of the gametes, occurred without abnormalities and within the periods typical of Mytilus galloprovincialis larvae developing in a natural environment.

Summary

The use of hormonal preparations and their combinations stimulated spawning in Mytilus galloprovincialis, whereas the control mussels did not spawn at all during the investigation period. The activity of spawning during stimulation with hormonal preparations depends on the preparation being used.

Of the hormones tested by us, prephyson, folliculin, testosterone, progesterone, prednisolone, pituitrin and mammophysin can be used as spawning stimulants, while hydrocortisone and chorionic gonadotropin can be used as hormones that prepare for spawning. The stimulating effect of the hormonal preparations depends on the time of the experiment. The experiments carried out in June—July demonstrated that prednisolone, as well as folliculin combined with prephyson, had a good stimulating effect. From August to October, good stimulation of spawning was induced by all the rest of the hormonal preparations studied, except for hydrocortisone and chorionic gonadotropin which only prepare for spawning.

Analysis of the preliminary data on two- and three-time stimulation of spawning in the same animals has shown that the optimal period between two artificially induced spawnings is 40-50 days, depending on the initial condition of the gonads.

(31

References

1. Problems of Hormonal Regulation of Reproduction in Some Species of Crustaceans and Bivalve Mollusks. Kaliningrad, Kaliningrad State University, 1982, 58 p. (Manuscript deposited at VINITI*, No. 0283 0020236)

2. Problems of Hormonal Regulation of Reproduction in Some Species of Crustaceans and Bivalve Mollusks. Kaliningrad, Kaliningrad State University, 1983, 79 p. (Manuscript deposited at VINITI*, No. 0284 0030728)

3. Malitsa N.A. Effect of the degeneration and irritation of the amygdalae on the sensitivity of the ovaries and uterus of infantile rats to hydrocortisone and desoxycorticosterone. Probl. endokrinol., 1979, v. 25, No. 2, pp. 45-50.

4. Mori K. Effect of estradiol-17b on sexual maturation and sexual transformation in the Japanese oyster Crassostrea gigas. 14th Pacific Science Congress. Khabarovsk, 1979. Com. F, Sect. 2a: Summaries of reports. Moscow, 1979, pp. 129-130.

5. Nikitina S.M. Steroidal Hormones of Invertebrates. Leningrad, Leningrad State University, 1982.

6. Nikitina S.M., Chibisova N.V. Effect of hormonal preparations on the relative variation rate of organ mass in two species of mussels. Third All-Union Conference on the Problems Concerning the Rational Use of Commercial Invertebrates. Kaliningrad, 1982: Summaries of reports, pp. 131-132.

7. Nikitina S.M., Kondratenko A.S., Savchenko O.N., Chibisova N.V., Nikitina T.Ye. Effect of hormonal preparations on the salt tolerance of the White Sea mussel. Third All-Union Conference on the Problems Concerning the Rational Use of Commercial Invertebrates. Kaliningrad, 1982, pp. 109-110.

8. Khotimchenko Yu.S. Effect of choriogonin on the oogenesis of sea urchins. All-Union Scientific Conference on the Use of Commercial Invertebrates for Food, Feed and Industrial Purposes. Odessa: Summaries of reports. Moscow, 1977, 101 p.

9. Khotimchenko Yu.S. Stimulation of oogenesis in sea urchins with chorionic gonadotropin. Second All-Union Conference on Shelf Biology. Sevastopol, 1978: Summaries of reports, Part 2. Kiev, 1978, pp. 118-119.

10. Endocrine Regulation of Reproduction in Aquatic Animals. Kalingrad, Kaliningrad State University, 1981, 90 p. (Manuscript deposited at VINITI, No. 0282 1017825)

14. Takahashi N. Effect of a steroid injection on the testes in Asterina pectinifera. Nippon suisan gakkaishi, Bull. Jap. Soc. Sci. Fish., 1982, No. 48, pp. 513-515.

*All-Union Institute of Scientific and Technical Information (of the State Committee of the Council of Ministers, USSR, for Science and Technology and of the Academy of Sciences, USSR) - transl.

1. Вопросы гормональной регуляции размножения отдельных видов ракообразных и двустворчатых моллюсков. - Калининград: Калининградский государственный университет, 1982. - 58 с. / Рукопись депонированная в ВИНИТИ, № 0283 0020236 .

2. Вопросы гормональной регуляции размножения отдельных видов ракообразных и двустворчатых моллюсков. - Калининград: Калининградский государственный университет, 1983. - 79 с. / Рукопись депонированная в ВИНИТИ, № 0284 0030728 .

3. Малица Н. А. Влияние разрушения и раздражения миндалевидных ядер на чувствительность яичников и матки инфантильных крыс к гидрокортизону и дезоксикортикостерону // Проблемы эндокринологии. - 1979. - Т. 25. - № 2. - С. 45-50.

4. Мори К. Действие эстрадиола - 17β на половое созревание и превращение пола у японской устрицы Crassostrea gigas // 14-й Тихоокеанский научный конгресс. Хабаровск. 1979. Ком. F. Секц.2а: Тезисы докладов. - М., 1979. - С. 129-130.

5. Никитина С. М. Стероидные гормоны беспозвоночных животных. - Л.: ЛГУ, 1982.

6. Никитина С. М., Чибисова Н. В. Влияние гормональных препаратов на относительную скорость изменения массы органов двух видов мидий // Третья Всесоюзная конференция по проблемам рационального использования промысловых беспозвоночных. Калининград, 1982: Тезисы докладов. - Калининград, 1982. - С. 131-132.

7. Влияние гормональных препаратов на соленостную толерантность беломорской мидии / С.М.Никитина, А.С.Кондратенко, О.Н.Савченко, Н.В.Чибисова, Т.Е.Никитина // Третья Всесоюзная конференция по проблемам рационального использования промысловых беспозвоночных. - Калининград, 1982: Тезисы докладов. Калининград, 1984. - С. 109-110.

8. Хотимченко Ю. С. Действие хориогонина на оогенез морских ежей // Всесоюзная научная конференция по использованию промысловых беспозвоночных на пищевые, кормовые и технические цели. Одесса: Тезисы докладов. - М., 1977. - 101 с.

9. Хотимченко Ю. С. Стимуляция оогенеза у морских ежей хорионическим гонадотропином // Вторая Всесоюзная конференция по биологии шельфа. Севастополь, 1978: Тезисы докладов. Ч. 2. - Киев, 1978. - С. 118-119.

10. Эндокринная регуляция размножения водных животных. - Калининград: Калининградский государственный университет, 1981. - 90 с. / Рукопись, депонированная в ВИНИТИ № 0282 1017825 .

11. Bomirski A., Klek-Kawinska E. Stimulation of oogenesis in the sand shrimp, Crangon crangon. Gen and Comp. Endocrinol., 1976, vol. 30, N 3, p.p. 239-242.

12. Cenovic M. G. Effet stimulant des gonadotropines de Mammiferes sur la reproduction des Daphnies. C.R. Acad. Sci., Paris, 1954, vol. 239, p.p. 722-724.

13. Kulkarni A. B., Utkar V. N., Kulkarni D. B. The effect of cortisone on haemolymph glucose level and tissue in the fresh water prosobranch snail Viviparus bengalensis (Lamark). J. Adv. Zool. 1981, vol. 2, N 2, p.p. 89-93.

14. Takahashi N. Действие инъекции стероидов на семенники Asterina pectinifera. Nippon suisan gakkaishi, Bull. Jap. Soc. Sci. Fish., 1982, N 48, p.p. 513-515.